

**REMARKS****I. Status of the Claims**

Claims 13-30 are pending.

Claims 13, 17-19, 21, 23-24 are amended.

Claim 30 is added.

**II. Comparison of Claims filed in the Amendment and Response to the Office Action Mailed June 3, 2002 Versus Claims Originally Filed.**

<b>PREVIOUS CLAIMS</b> <b>(In the Amendment and Response to the Office Action Mailed June 3, 2002)</b>	<b>ORIGINAL CLAIMS</b>
<p>13. A high throughput method for determining whether a compound or a mixture of compounds is suitable for intended use as a drug or a natural product, said method comprising:</p> <p>(a) placing a first solution comprising biological material having higher molecular weights than the compound or the mixture of compounds, into an ultrafiltration chamber, said chamber comprising a membrane with pore sizes that will not allow passage of the biological material out of the chamber;</p> <p>(b) placing the compound or the mixture of compounds into the ultrafiltration chamber, said chamber comprising a membrane with pore sizes that allow passage of the compound or the mixture of compounds out of the chamber;</p> <p>(c) providing a continuous flow of a supportive solution to the ultrafiltration chamber that</p>	<p>1. A method for determining whether a compound from a sample has predetermined characteristics that would make it suitable for a specific purpose, said purpose comprising drug development and screening for metabolic parameters, said method comprising:</p> <p>a. obtaining a biological material in a first solution or suspension;</p> <p>b. inducing a flow of a supportive solution through the first solution or suspension;</p> <p>c. adding the sample to the continuous flow of the supportive solution;</p> <p>d. reacting the biological material in the first solution or suspension with the compound in the sample to provide metabolites, or to assess permeability and bioavailability;</p> <p>e. washing the results of the reacting between the biological material in the first solution and the compound in the sample through an ultrafiltration</p>



<p align="center"><b>PREVIOUS CLAIMS</b></p> <p align="center"><b>(In the Amendment and Response to the Office Action Mailed June 3, 2002)</b></p>	<p align="center"><b>ORIGINAL CLAIMS</b></p>
<p>facilitates reactions between the biological material and the compound or the mixture of compounds to produce products of the reactions wherein the ultrafiltration chamber allows passage of the products out of the chamber to form a second solution, but does not allow passage of the biological materials;</p> <p>(d) analyzing the second solution comprising the products of the reactions between the biological material and the compound or the mixture of compounds, to determine whether the compound or any of the mixture of compounds is suitable for use as a drug or natural product.</p>	<p>membrane to form a second solution; and</p> <p>f. analyzing the second solution to determine whether the compound in the sample has the predetermined characteristics, wherein the predetermined characteristics are selected for the group consisting of functioning as a substrate for an enzyme, showing desirable rates of enzymatic catalysis, showing desirable rates of cell permeability or transport, and showing enzymatic activation to reactive or toxic metabolites.</p> <p>9. The method of claim 1, wherein the ultrafiltration membrane has pore sizes that allow the sample molecules to pass through but not the biological material.</p>
<p>14. The method of claim 13, wherein the biological material is selected from a group consisting of a protein, a peptide, an oligonucleotide, an oligosaccharide, a microsome, a cell, a tissue, an enzyme, DNA and RNA.</p>	<p>2. The method of claim 1, wherein the biological material is selected from a group consisting of a protein, a peptide, an oligonucleotide, an oligosaccharide, a microsome, a cell, a tissue, an enzyme, a receptor, DNA and RNA.</p>
<p>15. The method of claim 13, wherein the compound or mixture of compounds is selected from the group consisting of a natural product, a combinatorial library, a drug, a drug mixture, a xenobiotic compound, a mixture of xenobiotic compounds, an endogenous compound, a mixture of natural products, and a mixture of endogenous compounds.</p>	<p>3. The method of claim 1, wherein the compound is selected from the group consisting of a natural product, a combinatorial library, a drug, a drug mixture, a xenobiotic compound, a mixture of xenobiotic compounds, an endogenous compound, and a mixture of endogenous compounds.</p>
<p>16. The method of claim 13, wherein the</p>	<p>4. The method of claim 1, wherein the</p>



<p align="center"><b>PREVIOUS CLAIMS</b></p> <p align="center"><b>(In the Amendment and Response to the Office Action Mailed June 3, 2002)</b></p>	<p align="center"><b>ORIGINAL CLAIMS</b></p>
<p>supportive solution is selected from a group consisting of a buffer, a nutrient medium, or a combination thereof, said supportive solution maintaining the biological material in a state wherein the biological material reacts with a compound or mixture of compounds in the sample.</p>	<p>supportive solution is selected from a group consisting of a buffer, a nutrient medium, or a combination thereof, said supportive solution maintaining the biological material in a state wherein the biological material interacts with a compound in the sample.</p>
<p>17. The method of claim 16, wherein the supportive solution facilitates the reactions of the biological material with the first solution and facilitates the removal of compounds, or mixture of compounds and products of the reactions between the compound or the mixture of compounds and the biological material, by washing them through the ultrafiltration chamber into the second solution.</p>	<p>5. The method of claim 1, wherein the continuous flow facilitates the reacting of the biological material with the sample in the first solution or suspension and facilitates the removal of compounds from the sample by washing them through the ultrafiltration chamber into the second solution.</p>
<p>18. The method of claim 13, wherein the compound or the mixture of compounds is added by means of injection.</p>	<p>7. The method of claim 1, wherein the sample is added to the continuous flow by means of injection.</p>
<p>19. The method of claim 13, wherein the suitable conditions for reactions between the biological material in the first solution with the compound or the mixture of compounds, comprises mixing the sample with the biological material to achieve a homogeneous distribution of sample, controlling temperature to maintain function of the biological material, providing adequate concentration of sample and sufficient amount of biological material to facilitate analysis, providing sufficient time for interaction, and controlling atmospheric gases to maintain function of the biological material.</p>	<p>8. The method of claim 1, wherein the suitable conditions for reacting of the biological material in the first solution with the compound in the sample comprises mixing the sample with the biological material to achieve a homogeneous distribution of sample, temperature control to maintain function of the biological material, adequate concentration of sample and sufficient amount of biological material to facilitate analysis, sufficient time for interaction, and control of atmospheric gases (oxygen and carbon dioxide) to maintain function of the biological material.</p>
<p>20. The method of claim 13, wherein the analyzing of the second solution is by mass spectrometry.</p>	<p>10. The method of claim 1, wherein the analyzing of the second solution is by mass spectrometry.</p>
<p>21. The method of claim 13, wherein the products of the reactions comprise metabolites, glutathione adducts, and small molecules.</p>	
<p>22. The method of claim 13, wherein multiple chambers with ultrafiltration</p>	<p>12. The method of claim 1, wherein multiple chambers with ultrafiltration membranes are</p>



<b>PREVIOUS CLAIMS</b> <b>(In the Amendment and Response to the Office Action Mailed June 3, 2002)</b>	<b>ORIGINAL CLAIMS</b>
membranes are arranged in parallel with a single mass spectrometer for step d.	arranged in parallel with a single mass spectrometer for steps e and f.
23. A kit for analyzing a compound or mixture of compounds to determine if a compound or any of the mixture of compounds are suitable for use as a drug or natural product, by analyzing reaction products between biological material and the compound or mixture of compounds, said kit comprising in separate containers, (a) an ultrafiltration membrane with pore sizes that allow passage of the compound or mixture of compounds and reaction products, but not passage of the biological material, (b) a first solution containing the biological material, and (c) standards against which to compare analysis of the products of reactions between the first solution and the compounds or mixture of compounds to determine suitability as a drug or natural product.	11. A kit for analyzing a compound in a sample, to determine whether the compound has predetermined characteristics that would make it suitable for a specific purpose, said purpose comprising drug development and screening for metabolic parameters, said kit comprising in separate containers, an ultrafiltration membrane, a first solution containing a biological material, a buffer, a test solution, and a set of standard solutions with predetermined characteristics wherein the predetermined characteristics consist of functioning as a substrate for an enzyme, showing desirable rates of enzymatic catalysis, and showing desirable rates of cell permeability or transport, showing enzymatic activation to reactive or toxic metabolites.

### III. Support for the Claim Amendments

On page 3-4 of the Action, the examiner rejected new claims 13-23 under 35 U.S.C. § 112 first paragraph. Contrary to the examiner's assertions that there is no support for the claims, the following support from the specification for claim elements illustrates that the specification discloses the subject matter claimed.

Claim Element	Page/Line	Comments
high throughput	1/8-11	<b>high throughput</b> assays in combinatorial chemistry and drug development ( <i>emphasis added</i> ).
	2/11-14	"The method is a novel, <b>high-throughput</b> , on-line analysis of compounds added to a continuous flow system through biological materials in solution-that interact with the compounds. ' <b>High-throughput</b> ' is defined herein to be of the order of 1 metabolite (compound) processed per minute or more." ( <i>emphasis added</i> ).
supportive	3/1-3	"The <b>supportive solution</b> includes a buffer, a nutrient medium, or a combination thereof. The <b>supportive solution</b> is capable of



Claim Element	Page/Line	Comments
solution		maintaining the biological material in a state wherein the biological material can interact with a compound in the sample". ( <i>emphasis added</i> ).
suitable conditions	3/12-17	"The <b>suitable conditions</b> for interaction of the biological material in the first solution with the compound in the sample include mixing the sample with the biological material to achieve a homogeneous distribution, controlling temperature to maintain function of the biological material, providing adequate sample concentration and a sufficient amount of biological material to facilitate analysis, allowing sufficient time for interaction, and controlling atmospheric gases to maintain function of biological material". ( <i>emphasis added</i> ).
kit	3/23-27	"A <b>kit</b> for analyzing compounds in a sample includes, in separate containers, an ultrafiltration membrane, a first solution containing a biological material, a buffer, a test solution, a set of standard solutions with predetermined characteristics and a receptacle for the second solution that results from interactions of the compounds with the biological material". ( <i>emphasis added</i> ).
use as a drug	1/6-10 2/9-11 6/6-8 9/7-10	<p>"This invention relates to a high throughput, on-line, pulsed ultrafiltration method <b>useful for drug development</b> and screening for metabolic parameters, bioactivation and potential toxicity of compounds and molecules".</p> <p>"The present invention provides a method of determining whether a compound has predetermined characteristics that make it a candidate for <b>drug development</b> or a substrate for a particular enzyme".</p> <p>"A high throughput, on-line, pulsed ultrafiltration-mass spectrometric method has been developed to determine whether a compound has predetermined characteristics that would make it <b>suitable for a specific purpose, e.g. drug development</b>".</p> <p>"The compounds that may be screened include <b>drugs, drug candidates</b>, combinatorial libraries, natural products, pesticides, herbicides, other xenobiotic compounds and endogenous biological compounds". (<i>emphasis added</i>)</p>
natural product	2/30-31 9/7-10	<p>"The compound is generally a candidate for drug development produced by combinatorial chemistry or a <b>natural product</b>".</p> <p>"The compounds that may be screened include <b>drugs, drug candidates</b>, combinatorial libraries, <b>natural products</b>, pesticides, herbicides, other xenobiotic compounds and endogenous biological compounds". (<i>emphasis added</i>).</p>
mixture of	6/31-34	"A sample with a <b>compound</b> (or molecule(s) or a <b>mixture of compounds</b> ) to be tested is joined with the continuous stream of a



Claim Element	Page/Line	Comments
compounds	8/23-25  8/28-32  15/5-9  Example 7 20/10-12	<p>supportive solution, as a pulse. Preferably the sample to be tested is <b>injected</b> into the stream to interact with the biological material in the first solution”.</p> <p>Xenobiotic <b>compounds</b> (either individually or as <b>mixtures</b>) are <b>injected</b> through the ultrafiltration chamber and their elution profiles are recorded using mass spectrometric detection.</p> <p>Because each pulsed ultrafiltration mass spectrometric determination of bioavailability requires approximately 1 hr, and <b>mixtures of compounds</b> may be measured simultaneously, this approach is much faster than previous methods that may require approximately one day each to complete.</p> <p>For example, the throughput may be increased 10-fold by <b>injecting mixtures containing 10 compounds</b> at a time.</p> <p>Pulsed ultrafiltration mass spectrometric screening may be used on line for <b>high throughput</b> detection of <b>glutathione adducts</b> for rapid toxicity screening of compounds or <b>compound mixtures</b>.” (<i>emphasis added</i>)</p>
supportive solution facilitates the removal of compounds, or mixture of compounds	3/4-6  2/20-23	<p>“The continuous flow facilitates the interaction of the compound(s) with the biological material and then <b>facilitates the removal of the compounds and metabolites thereof</b> by washing them through the ultrafiltration membrane and into the second solution for analysis”.</p> <p>“maintaining a continuous flow of a <b>supportive solution</b> through the first solution or suspension”. (<i>emphasis added</i>).</p>
glutathione adducts	5/4-5  5/12-16  5/17-18	<p>“FIG. 7 shows results of screening for formation of <b>glutathione adducts</b> as indicators of toxic (electrophilic) metabolites using pulsed ultrafiltration-mass spectrometry.</p> <p>Oxidation of butyldimethyl phenol by cytochromes P450 produced a reactive quinone methide intermediate which either reacted with water to form the oxidation product detected at m/z 193, or reacted with <b>glutathione to form the adduct</b> detected at m/z 482. As expected, <b>glutathione adducts</b> were observed only in experiments containing both glutathione and NADPH.</p> <p>FIG. 8 illustrates constant neutral loss LC-MS-MS analysis of the <b>glutathione adducts</b> of 3-methylindole formed during pulsed ultrafiltration toxicity screening”. (<i>emphasis added</i>).</p>
metabolites	8/15-19	“The on-line pulsed ultrafiltration-mass spectrometric observation of reactive <b>metabolites or glutathione adducts</b> formed from reactive <b>metabolites</b> indicates that a particular substrate forms potentially



Claim Element	Page/Line	Comments
	9/24-26	toxic <b>metabolites</b> . <b>Metabolites</b> formed in the ultrafiltration chamber are washed out by the buffer solution being pumped through the ultrafiltration membrane and are detected by the on-line electrospray mass spectrometer 4". ( <i>emphasis added</i> ).
small molecules	1/13-16  27/17-18	"Combinatorial chemistry, a new approach to the identification and optimization of drug leads in medicinal chemistry, has been enormously successful in synthesizing large number of compounds for pharmacological screening and testing (Gordon et al., 19%; Thompson and Ellman, 1996).  Thompson, L. A. and Ellman, J. A.: Synthesis and applications of <b>small molecule</b> libraries. <i>Chem. Rev.</i> 29:132-143 (1996)". ( <i>emphasis added</i> ).
pore sizes	3/18-19  7/2-10  13/27-30	"The ultrafiltration membrane <b>pore sizes</b> allow the sample to pass through but not the biological material".  "The <b>pore size</b> of the membrane can, however, be closer to retain molecules of whatever molecular weight that is suitable for a particular application. <b>Pore sizes</b> may range from those allowing only very low molecular weight products to pass through, <i>e.g.</i> acetone or benzene (50-100D) to larger molecules in the 12,000-20,000D range <i>e.g.</i> enzymes, myoglobin, to very large molecules (100,000-million D). The <b>pore size</b> must be small enough to prevent the biological material from passing through, <i>e.g.</i> a 10,000 molecular weight cut-off pore size prevents adenosine deaminase from passing through and a 100,000 molecular weight cut-off membrane prevents microsomes from passing through.  Because of the large size of cells compared to enzymes, much larger <b>pore sizes</b> may be used for the ultrafiltration membrane and molecular weight cutoffs of 500,000 or 1,000,000 daltons work well with low back pressure". ( <i>emphasis added</i> ).
NADPH	1/31-32  4/14-15 (FIG. 2) 4/19-20 (FIG. 5) 4/23-25 (FIG. 5)	"The fastest current method to assess drug metabolism utilizes hepatic microsomes, which are incubated in a test tube with a drug and the cofactor <b>NADPH</b> .  <b>NADPH</b> was added to the continuous flow.  The control experiment was identical except that no <b>NADPH</b> was present.  Mass spectrometric analysis performed for 2 minutes, of effluent from the parallel control incubation (containing <b>NADPH</b> and inactive microsomes) showed no metabolites of imipramine.



Claim Element	Page/Line	Comments
	5/7-9 (FIG. 7)	The substrate, butyldimethyl phenol was injected along with <b>NADPH</b> and glutathione as cofactors. As expected, glutathione adducts were observed only in experiments containing both glutathione and <b>NADPH</b> .
	7/20-23	Any necessary cofactors, such as <b>NADPH</b> (for cytochrome P-450 oxidation), UDPGA for glucuronyl transferase, or glutathione for glutathione transferase, are either included in the continuous phase or co-injected with the xenobiotic substrate". ( <i>emphasis added</i> ).

On page 5 of the Action, the examiner stated that the applicants may show possession of the full scope of the claimed invention by way of representative examples. The application discloses 8 illustrative examples that include high throughput metabolic screening, toxicity screening using ultrafiltration mass spectrometry, pulsed ultrafiltration-mass spectrometry analysis of glutathione adducts and high throughput toxicity screening. The specification discloses analysis of small molecules such as pentoxyresorufin in assaying cytochrome P450 2B-catalyzed O-dealkylation activity (page 16, Example 2), high throughput metabolite screening of imipramine, chlorpromazine, quinidine and narigenin (page 17, Example 3; FIG. 2), analysis of glutathione adduct formation from reactive drugs (page 19, Example 6; FIG. 8) and other applications of the methods claimed in the present application. Thus the specification discloses sufficient representative examples to show possession of the claimed invention.

#### IV. Claims 13-22 Satisfy 35 U.S.C. § 112 Second Paragraph Requirements

On page 5 of the Action, the examiner rejected claims 13-22 for being indefinite. Amended claims 13, 17-19, and 23-24 have proper antecedent basis and hence the § 112 second paragraph rejection should be withdrawn. On page 5 of the Action, the examiner stated that claim 21 is indefinite for lacking some essential elements. Claim 21 is amended and new claim 30 is added. Therefore, the § 112 second paragraph rejection should be withdrawn for claim 21.

#### V. Other Issues

This case has been pending since 1999. It has gone through 3 examiners and now a 4<sup>th</sup>, and 3 supervisors. There have been 2 interviews. Every time applicants act on a suggestion by an examiner



or a supervisor, and files an amendment, in the next Action the examiner appears to change his mind without sufficient explanation.

An RCE and a Preliminary Amendment was filed on the basis that claims were then in condition for allowance. Instead, the examiner withdrew some of the claims (24-29) and rejected others (13-23) as lacking support. This was despite applicant providing the support from the specification in the amendment filed with the RCE. The extent of the support expanded herein also shows possession of the invention.

Claims 24-29 do not have language that was not previously examined, so it is inappropriate to restrict them out at this point. Claim amendments result from suggestions by the examiner and the supervisor.

#### **VI. Summary and Conclusion**

Applicants request allowance of claims 13-30. Applicants request a telephone interview to put claims in condition for allowance.

Please contact applicants' representative if you have any questions.

No other fees are believed due at this time, however, please charge any deficiencies or credit any overpayments to deposit account number 12-0913 with reference to our attorney docket number (21419/90386).

Respectfully submitted,



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